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A Mild Magnesium Deprivation Affects Calcium Excretion But Not Bone Strength and Shape, Including Changes Induced by Nickel Deprivation, in the Rat*,†,‡

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ABSTRACT

An experiment was performed to determine the effect of a mild magnesium deprivation on calcium metabolism and bone composition, shape, and strength in rats, and whether nickel deprivation exacerbated or alleviated any changes caused by the magnesium deprivation. Weanling male rats were assigned to groups of 10 in a factorial arrangement, with variables being supplemental nickel at 0 and 1 mg/kg and magnesium at 250 and 500 mg/kg of diet. The basal diet contained about 30 ng Ni/g. Urine was collected for 24 h during wk 8 and 12, and rats were euthanized 13 wk after dietary treatments began. Mild magnesium deprivation decreased the urinary excretion of calcium and increased the tibia concentration of calcium but did not affect femur shape or strength (measured by a three-point

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bending test). Dietary nickel did not alter these effects of magnesium deficiency. Nickel deprivation increased the urinary excretion of phosphorus and the femur strength variables maximum force and moment of inertia. Strength differences might have been the result of changes in bone shape. Magnesium deprivation did not alter the effects of nickel deprivation on bone. The findings indicate that a mild magnesium deficiency affects calcium metabolism but that this does not markedly affect bone strength or shape, and these effects are not modified by dietary nickel. Also, nickel deprivation affects phosphorus metabolism and bone strength and shape; these effects apparently are not caused by changes in magnesium metabolism or utilization.

Index Entries: Magnesium; nickel; bone; calcium; minerals; trace elements.

INTRODUCTION

Epidemiological studies have linked low dietary intakes of magnesium to decreased bone mass and osteoporosis in humans *(1).* However, very few studies oriented toward determining the effect of long-term, marginal, or mildly deficient intakes of magnesium (similar to that found in the general population) on calcium metabolism and bone biomechanical properties have been reported. As a result, the significance of low dietary intakes of magnesium in bone loss and osteoporosis is uncertain. Moreover, because of experimental flaws, most of the limited studies examining the effect of magnesium on postmenopausal osteoporosis have not provided persuasive evidence that magnesium plays a role in the pathogenesis or treatment of the disease *(2).* This lack of credible evidence was apparently one of the reasons that the Food and Nutrition Board *(3)* recommended further studies be performed to determine the relationships between various magnesium intakes on health outcomes such as altered bone turnover and osteoporosis.

Magnesium deficiency unquestionably can affect calcium metabolism and bone growth and maintenance. A severe magnesium deficiency (generally less than 10% of the requirement) in experimental animals results in decreased bone growth, osteopenia, and increased skeletal fragility *(4–6).* Recent findings indicate that these changes are caused by decreased osteoblast number, increased osteoclast number, and loss of trabecular bone *(1,6).* Magnesium deficiency in chicks, in contrast to severe deficiency in rats, diminished bone resorption without changing bone formation; this resulted in increased cortical thickness instead of osteopenia *(7,8).* It should be noted that magnesium deficiency causes hypercalcemia in the rat, but causes hypocalcemia in the chick (and other mammalian species) *(4).* This suggests that parathyroid action is not markedly impaired by magnesium deficiency in the rat *(4).*

In humans, a severe magnesium deficiency, as found with losses of magnesium through the gastrointestinal tract or the kidney because of

disease or drugs, impairs parathyroid metabolism and causes hypocalcemia *(5).* However, magnesium deprivation similar to that more likely to be found in the general population (e.g., 50% of the estimated average requirement [EAR] established by the Food and Nutrition Board) *(3)* apparently has an inconsistent effect on parathyroid secretion and does not markedly affect serum calcium *(9).* Thus, a mild magnesium deficiency in rats might be an acceptable experimental model to study the type of magnesium deprivation most likely to occur in humans. However, there are very few reports of studies determining the effect of mild magnesium deprivation on bone characteristics in rats. Rats fed 50% of the magnesium requirement for 7 mo exhibited decreased humerous trabecular bone mineral density and content, but, like the magnesium-deficient chick, it had increased cortical bone mineral density and thickness *(10).* Surprisingly, these bone changes apparently did not result in any significant change in the biomechanical properties because bone-breaking variables of the femur and vertebra were not significantly affected by magnesium deficiency.

Regardless of the species or severity, magnesium deficiency affects calcium metabolism. Surprisingly, most studies indicate that deficiency enhances, not impairs, calcium absorption and retention. For example, severe magnesium deficiency (~5% of the requirement) significantly increased calcium absorption and balance in rats *(11).* Mature rats fed ~50% of the magnesium dietary requirement for 4–10 wk exhibited increased calcium absorption and balance *(12).* Humans fed only 3% of the estimated average magnesium requirement retained more calcium and excreted less in the urine *(13).* Postmenopausal women fed 50% of the estimated average magnesium requirement excreted less urinary calcium and tended to have increased calcium balance *(14).* Thus, the lack of calcium entering and being retained in the body apparently is not the cause of impaired bone health in severe magnesium deficiency. Decreased urinary excretion and increased retention of calcium during a marginal or mildly deficient magnesium intake does not preclude that such an intake eventually leads to some impairment in bone health based on the finding that trabecular bone is decreased by such a deficiency *(10).*

An element that varies in food in a manner similar to magnesium is nickel. Among the first reported signs of nickel deprivation were changes in calcium metabolism and bone structure and composition. These signs included thickened legs, swollen hock joints, and a reduced tibia length/width ratio in chicks *(15),* increased renal excretion of calcium and decreased calcium concentration in the skeleton of mini-pigs *(16),* and an increased magnesium concentration in femurs of rats *(17).* The findings suggest that nickel deprivation has the opposite type of effects on calcium metabolism from that of magnesium deprivation, but a similar effect on bone structure. The change in bone magnesium in nickel deprivation suggests that an interaction between nickel and magnesium might alter the effect of the deprivation of each of these elements on bone characteristics.

Based on the preceding findings, an experiment was performed in which one aspect was to confirm that a mild magnesium deficiency does not markedly affect bone strength and structure, but changes bone composition and calcium metabolism, and to determine whether these effects are altered by nickel deprivation.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing about 48 g were assigned to 4 groups of 10 and fed diets in a factorial arrangement, with variables being supplemental nickel at 0 and 1 mg/kg and magnesium at 250 and 500 mg/kg diet. The rats were housed individually in plastic cages in laminar airflow racks. The racks were located in a room maintained at 23°C and 50% relative humidity with a 12-h light–dark cycle. The rats had free access to food and deionized drinking water (Super Q; Millipore, Bedford, MA). Absorbent paper under the false-bottom cages was changed daily. Animals were weighed and provided clean cages weekly.

The composition of the basal diet is shown in Table 1. The nickel supplement mix contained 0.4050 g $NiCl_2 \cdot 6H_2O$ and 99.595 g sucrose; 1 g of the mix per kilogram provided 1 mg Ni/kg diet. The basal diet was determined to contain about 30 ng Ni/g by using a graphite furnace atomic absorption spectrometric method following a wet-ashing procedure *(18).* Standard reference material #1515 Apple Leaves (National Institute of Standards and Technology, Gaithersburg, MD) was used for quality control. The diets were not pelleted and were stored at –16°C in tightly capped plastic containers.

During wk 8 and 12 of the experiment, rats were placed individually in metabolic cages with free access to deionized water but not food while urine was collected for 24 h in plastic test tubes kept on ice. After 13 wk on their respective diets, rats were weighed and anesthetized with ether for the collection of blood from the vena cava with a heparin-coated syringe and needle. After euthanasia by decapitation, the kidney, leg muscle, femurs with some attached flesh, tibias with all flesh removed, and plasma were collected and stored at –70°C until analyzed.

The study was approved by the Animal Care Committee of the Grand Forks Human Nutrition Research Center, and the lawfully acquired animals were maintained in accordance with the NIH guidelines for the care and use of laboratory animals.

Commercially available assay kits (Sigma, St. Louis, MO) were used to determine plasma triglycerides (Triglyceride INT 10 Kit #336-10), glucose (PGO enzyme #510A), and alkaline phosphatase (104 Phosphatase substrate #104-105). Commercially available assay kits (Cayman Chemical Co., Ann Arbor, MI) also were used to determine urine prostaglandin E_2 (Bicyclo Prostaglandin E_2 EIA Kit, cat. no. 514531) and nitrate (Nitrate/Nitrite Colorimetric Assay Kit #780001). For mineral analysis, tibias were cleaned

Table 1
Composition of the Basal Diet

Ingredient	g/kg
Vitamin-free casein	165.00
Corn starch	150.00
Sucrose	438.33
Cellulose (alphacel)	80.00
Palm oil	100.00
l-Cystine	5.00
l-Lysine	5.00
l-Tryptophan	0.50
l-Isoleucine	5.00
Choline chloride	0.75
MgO*	0.42
Vitamin Mix†	5.00
Mineral Mix – 1‡	27.00
Mineral Mix -2§	18.00
Total	1000.00

* An additional 0.42 g of MgO was added to the diet to make the diet with 500 mg Mg/kg; sucrose was reduced accordingly.

† The vitamin mix contained (in mg): *dl*-α-tocopherol acetate (300 IU/g), 120; retinyl palmitate (250,000 IU/g), 16; vitamin D_3, (400,000 IU/g), 2.5; phylloquinone, 1; biotin, 0.2; folic acid, 1; niacin, 30; calcium pantothenate, 16; riboflavin, 6; thiamine, 6; pyridoxine·HCl, 8; vitamin B_{12} (0.1% in mannitol), 50; and sucrose, 4743.3.

‡ Mineral mix 1 contained (in g): $CaCO_3$, 12.5; NaCl, 12.5; and KCl, 2.

§ Mineral mix 2 contained (in mg): KH_2PO_4, 8800; $CuSO_4 \cdot 5H_2O$, 20; $MnSO_4 \cdot 4H_2O$, 165; ZnO, 10; KI, 0.5; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.3; Na_2SeO_3, 0.6; $Na_2HAsO_4 \cdot 7H_2O$, 0.5; H_3BO_3, 6; $Cr(C_2H_3O_2)_3 \cdot 3H_2O$, 2; NaF, 6; $Na_2SiO_3 \cdot 9H_2O$, 100; NH_4VO_3, 0.5; $Fe_2(SO_4)_3$, 125; and sucrose, 8763.6.

to the periosteal surface with cheesecloth and lyophilized before wet ashing *(18)*. Urine was centrifuged and analyzed without digestion or dilution. All minerals were determined by inductively coupled argon plasma atomic emission spectroscopy except nickel, which was determined by using a graphite furnace atomic absorption spectrometric method *(18)*. Standard reference material #1515 Apple Leaves (National Institute of Standards and Technology, Gaithersburg, MD) and Urine Toxicology Control (UTAK Laboratories, Valencia, CA) were used for quality control.

Bone strength variables were determined on right femurs after thawing and removing the remaining flesh. A custom-designed and custom-built apparatus that performed a three-point bending test the same as

commercially available machines was used to determine bone-breaking variables. The length between fulcra and point-of-force application was determined by femur length. The point of force (crosshead) was centered over the greatest possible distance between the two fulcra. The femur was placed in a stable position with the ventral side up and the knee joint to the left while facing the instrument. The rate of deformation was a constant 5 mm/min. Descriptions of the terms used for the assessment of bone strength and elasticity have been described *(19)*. Briefly, the definitions of the terms are as follows. Maximum force is the force in Newtons needed to break the bone. Stress is the maximum force per unit cross-sectional area at the breaking point. Strain is a ratio of bending distance before breaking and original length; modulus of elasticity (indicator of flexibility) is stress divided by strain. Area moment of inertia is the effect of bone geometry on resistance to bending. Bending moment is force times length between the fulcra. The outside and inside lateral widths and ventral depth measurements at the breaking point were determined by using an electronic digital caliper.

Data were statistically compared by using two-way analysis of variance (SAS/STAT, Version 8.02; SAS Institute, Inc., Cary, NC) followed by Tukey's contrasts when appropriate. Outliers more than two standard deviations from the mean were not included in the analysis. A p-value of <0.05 was considered significant.

RESULTS

Neither mild magnesium deprivation nor nickel deprivation affected final body weight (Table 2). This was not unexpected because growth usually is unaffected by these dietary treatments. Thus, tissue concentrations of nickel and magnesium are often used as indicators of status. In the kidney, the concentration of nickel was decreased by nickel deprivation and the concentration of magnesium was decreased by magnesium deprivation, with the effect most marked in the nickel-deprived rats (Table 2). Other variables that have been shown to respond to dietary magnesium and nickel also are shown in Table 2. The concentration of triglycerides in plasma was increased by nickel deprivation and the excretion of nitrate in the urine during wk 12 was increased by magnesium deprivation. Neither plasma glucose nor blood hemoglobin concentrations were affected by the dietary treatments.

The effect of the dietary treatments on the urinary excretion of minerals at 8 and 12 wk of the experiment are shown in Tables 3 and 4, respectively. At 8 wk, significant changes were few. Magnesium deprivation decreased the urinary excretion of calcium regardless of dietary nickel and the excretion of magnesium in the nickel-deprived rats. During wk 12 of the experiment, additional significant changes in urinary minerals occurred. Magnesium deprivation decreased the urinary excretion of cal-

Table 2
Effect of Dietary Magnesium and Nickel on Indicators of Nickel and Magnesium Status

Diet		Final	Kidney		Urine	Plasma		Blood
Ni	Mg	Weight	Ni	Mg	Nitrate	Glucose	Triglycerides	Hemoglobin
mg/kg	mg/kg	g	ng/g	µg/g	U‡	mg/dL	mg/dL	g/dL
0*	250	395	52	772a†	25.5	171	92	12.49
1	250	382	77	800b	26.2	161	56	12.30
0	500	385	52	822b	23.5	181	95	12.27
1	500	391	68	814b	20.7	166	69	12.37
Analysis of Variance – P values								
Nickel		0.73	0.0008	0.12	0.50	0.13	0.04	0.76
Magnesium		0.96	0.42	0.0001	0.03	0.36	0.58	0.60
Ni x Mg		0.39	0.42	0.009	0.29	0.76	0.75	0.31
Pooled SD		33	17	19	5.0	24	44	0.44

* The basal diet contained 30 ng Ni/g.

† Values not followed by the same superscript are significantly different according to Tukey's contrasts.

‡ Units are nmoles/µmoles creatinine. Urine was collected during wk 12 of the experiment.

cium and magnesium. Nickel deprivation increased the excretion of phosphorus and decreased the excretion of iron and nickel in urine. An interaction between magnesium and nickel affected the urinary excretion of copper and zinc. Urinary copper was increased by nickel deprivation in the magnesium-deprived rats but not in the magnesium-adequate rats. Urinary zinc appeared to be decreased by magnesium deprivation in the nickel-supplemented rats but not in the nickel-deprived rats.

The dietary magnesium and nickel treatments affected tibia mineral composition differently (Table 5). Magnesium deprivation significantly increased the calcium and manganese concentrations and tended ($p < 0.07$) to increase the phosphorus concentration in tibia. Nickel deprivation increased the concentration of magnesium in tibia, with the effect most marked in the magnesium-adequate rats. Nickel deprivation increased the concentration of copper in magnesium-adequate but not in magnesium-deficient rats. Neither nickel nor zinc concentrations in tibia were affected by the dietary treatments.

Surprisingly, although magnesium deprivation affected tibia mineral composition, it did not affect femur shape (Table 6) or strength (Table 7).

Table 3
Effect of Dietary Nickel and Magnesium on the Urinary Excretion of Minerals at 8 wk

Diet		Urine				
Ni	Mg	Ca	P	Mg	Ni	Zn
ng/kg	mg/kg	µg/hr	µg/hr	µg/hr	ng/hr	µg/hr
0*	250	15	384	14[a†]	4.2	0.19
1	250	17	511	47[b]	8.4	0.21
0	500	23	419	78[c]	8.1	0.15
1	500	36	372	55[bc]	9.0	0.24
Analysis of Variance – P values						
Nickel		0.07	0.52	0.52	0.11	0.22
Magnesium		0.002	0.39	0.0001	0.16	1.00
Ni x Mg		0.16	0.15	0.0004	0.29	0.45
Pooled SD		12	180	22	4.9	0.14

* The basal diet contained 30 ng Ni/g.
† Values not followed by the same superscript are significantly different according to Tukey's contrasts.

An interaction between magnesium and nickel significantly affected the distal length (length between the breaking point and the knee joint) and the distal length/total length ratio. Nickel deprivation tended to increase these variables in the magnesium-adequate rats. Nickel deprivation decreased the outside ventral depth and tended to decrease ($p < 0.06$) the inside ventral depth measured at the point where the break occurred in the bone-breaking procedure. Nickel deprivation also decreased the moment of inertia and the maximum force needed to break the bone (Table 7). The strength variables stress, bending moment, and modulus of elasticity were not affected by the dietary treatments.

Other variables measured that might be related to changes in bone and calcium metabolism are shown in Table 8. Kidney and muscle calcium concentrations and plasma alkaline phosphatase activity were not significantly affected by the dietary treatments. Urinary prostaglandin E_2 excretion (ng/h) in the wk 12 collection was significantly higher in nickel-deprived than nickel-supplemented rats fed the magnesium-deficient diet. Because average amount of urine collected from nickel-deprived rats (1.48 and 1.27 mL/h for magnesium-deficient and magnesium-ade-

Table 4
Effect of Dietary Nickel and Magnesium
on the Urinary Excretion of Minerals at 12 wk

Diet		Urine						
Ni	Mg	Ca	P	Mg	Ni	Zn	Cu	Fe
mg/kg	mg/kg	µg/hr	µg/hr	µg/hr	ng/hr	µg/hr	µg/hr	ng/hr
0*	250	13	714	37	1.5	0.20	0.109[a†]	11.8
1	250	12	567	29	4.5	0.14	0.065[b]	24.5
0	500	21	670	51	1.7	0.17	0.077[b]	12.9
1	500	24	540	54	6.1	0.22	0.075[b]	22.5
		Analysis of Variance – P values						
Nickel		0.75	0.006	0.61	0.0001	0.87	0.002	0.002
Magnesium		0.0009	0.46	0.0002	0.22	0.31	0.12	0.89
Ni x Mg		0.37	0.86	0.24	0.32	0.04	0.004	0.64
Pooled SD		8.7	147	14	2.2	0.08	0.021	10.4

* The basal diet contained 30 ng Ni/g.
† Values not followed by the same superscript are significantly different according to Tukey's contrasts

quate rats, respectively) was much higher than that collected from nickel-supplemented rats (0.73 and 1.07 mL/h for magnesium-deficient and magnesium-adequate rats, respectively), the concentration of prostaglandin E_2 was lower in urine from nickel-deprived than nickel-supplemented rats.

DISCUSSION

Decreased kidney nickel has been used as an indicator of nickel deprivation *(20).* Thus, based on the significant decrease in kidney nickel concentration, rats fed the diet containing about 30 ng/g were nickel deficient. Other nickel-responsive variables support this conclusion; these include decreased urinary excretion of nickel and increased plasma triglycerides. Increased plasma triglycerides have been found in other nickel-deficiency studies *(21,22).* Decreased plasma glucose and blood hemoglobin have also been reported to be signs of nickel deprivation *(23).* The lack of significant effect of nickel on plasma glucose might have been caused by the high sucrose content of the diet, which apparently increased plasma glucose

Table 5
Effect of Dietary Nickel and Magnesium on the Tibia (Dry Weight) Mineral Concentrations

Diet		Tibia							
Ni	Mg	Ca	P	Mg	Ni	Cu	Fe	Mn	Zn
mg/kg	mg/kg	mg/g	mg/g	mg/g	ng/g	µg/g	µg/g	µg/g	µg/g
0*	250	224	118	2.67	178	0.438[ab†]	49.4	0.545	202
1	250	220	117	2.61	163	0.465[a]	47.9	0.551	202
0	500	215	115	2.91	175	0.470[a]	48.6	0.502	204
1	500	213	115	2.55	174	0.373[b]	57.4	0.462	210
Analysis of Variance – P values									
Nickel		0.27	0.51	0.01	0.72	0.06	0.33	0.38	0.46
Magnesium		0.01	0.07	0.25	0.86	0.10	0.25	0.002	0.18
Ni x Mg		0.69	0.61	0.06	0.75	0.002	0.17	0.25	0.38
Pooled SD		8	4	0.24	64	0.060	11.4	0.059	11

* The basal diet contained 30 ng Ni/g.
† Values not followed by the same superscript are significantly different according to Tukey's contrasts.

concentrations in all rats; glucose concentrations are often reported in the 120–140 range *(24,25).* Blood hemoglobin response to nickel deprivation has not been consistently found and might be affected by other dietary factors *(26);* thus, it probably is not a good indicator of nickel status.

Kidney magnesium has been used as an indicator of magnesium status *(27).* Magnesium deprivation decreased kidney magnesium in nickel-deprived rats. This suggests that the marginal magnesium status obtained in rats was slightly exacerbated by nickel deprivation. The decreases in urinary magnesium and calcium excretion and increase in urinary nitrate excretion supports the conclusion that rats fed 250 mg Mg/kg diet had a suboptimal magnesium status. Increased nitric oxide production, which leads to increased urinary nitrate, is one of the first signs of magnesium deprivation in the rat *(28),* and decreased urinary magnesium and calcium in response to magnesium deprivation has been reported for humans *(13,14).*

The change in the effect of the dietary treatments on urinary mineral excretions between 8 and 12 wk suggests that long-term feeding of nickel-deficient and moderately magnesium-deficient diets are required to see

Table 6
Effect of Dietary Nickel and Magnesium on Femur Shape at Breaking Point

Diet		Ventral depth		Lateral width		Length		Distal/
Ni	Mg	Inside	Outside	Inside	Outside	Total	Distal	Total
mg/kg	mg/kg	mm	mm	mm	mm	mm	mm	
0*	250	1.73	3.14	2.69	4.09	38.4	17.6	0.459
1	250	1.91	3.32	2.68	4.06	39.0	18.3	0.470
0	500	1.81	3.15	2.65	4.04	39.0	18.5	0.474
1	500	1.88	3.28	2.75	4.15	38.8	17.6	0.455
Analysis of Variance – P values								
Nickel		0.06	0.02	0.60	0.57	0.61	0.77	0.54
Magnesium		0.71	0.79	0.84	0.79	0.61	0.82	0.99
Ni x Mg		0.39	0.63	0.45	0.36	0.30	0.01	0.04
Pooled SD		0.18	0.17	0.21	0.20	1.06	0.83	0.021

* The basal diet contained 30 ng Ni/g.

significant responses to these dietary treatments. Thus, the termination of the experiment at 13 wk apparently was an appropriate time for termination. At this time, the magnesium deprivation apparently had an effect on calcium metabolism because it decreased the urinary excretion and increased the tibia concentration of calcium. Other long-term moderate magnesium-deprivation experiments have shown similar results. Riond et al. *(10)* found that rats fed 200 mg Mg/kg diet for 7 mo had increased calcium concentrations in tibia and vertebrae. A mild magnesium deprivation in humans increased the urinary excretion of calcium *(14).* The lack of an effect of moderate magnesium deprivation on urinary phosphorus excretion was also found by Bergstra et al. *(29).* The decrease in urinary calcium apparently did not result in soft tissue retention because neither kidney nor muscle calcium was increased by magnesium deprivation in the present experiment.

The reason for nickel deprivation increasing urinary phosphorus excretion is unclear, but it apparently was not caused by a changed deposition of phosphorus in bone. Nickel might be affecting the excretion of phosphorus by changing lipid metabolism or a signaling function. Nielsen et al. *(30)* reported that nickel deprivation decreased liver lipid-bound phosphorus in chicks. Stangl and Kirchgessner *(22)* found that

Table 7
Effect of Dietary Nickel and Magnesium on Femur Strength

Diet		Maximum	Moment	Stress	Bending	Modulus of
Ni	Mg	Force	Of Inertia		Moment	Elasticity
mg/kg	mg/kg	N*	mm^4	MPa†	N/mm	MPa†
0‡	250	148	5.50	146	500	8663
1	250	163	6.54	131	503	8197
0	500	145	5.48	136	485	8729
1	500	159	6.36	134	509	8395
Analysis of Variance – P values						
Nickel		0.01	0.02	0.19	0.43	0.37
Magnesium		0.53	0.81	0.61	0.79	0.77
Ni x Mg		0.94	0.84	0.33	0.54	0.88
Pooled SD		15	1.12	18	50	1306

* N = Newtons.
† MPa = MegaPascals.
‡ The basal diet contained 30 ng Ni/g.

nickel deprivation increased the concentration of serum phospholipids and changed the phosphatidylcholine and phosphatidylethanolamine distribution in liver fatty acids. Also, there are findings suggesting that nickel might have a function that can sensitize cyclic guanosine monophosphate-gated channels *(31)*.

Trace element metabolism or utilization apparently also was affected by the dietary treatments. Nickel deprivation increased the urinary excretion of copper in magnesium-deficient rats. In the tibia, the interaction between magnesium and nickel resulted in nickel deprivation increasing the concentration of copper in the tibia of magnesium-adequate rats. The bases for these changes are unclear, but both antagonistic and synergistic interactions between nickel and copper have been reported *(18,32–34)*. Also, a mild magnesium deprivation has been shown to increase copper absorption and the concentrations of copper in muscle, kidney, and liver in rats *(35)*. The decreased iron excretion in nickel-deprived rats might be reflecting an effect on iron metabolism; several studies indicate that dietary nickel affects the utilization of iron *(18,21,23,26)*. The changed urinary iron excretion, however, was not reflected by a change in the concentration of iron in the tibia and probably did not affect changes found in femur biomechanical characteristics.

Table 8
Effect of Dietary Nickel and Magnesium on Calcium and Bone Metabolism Variables

Diet		Kidney	Muscle	Urine		Plasma
Ni	Mg	Ca	Ca	Prostaglandin E_2		Alkaline Phosphatase
mg/kg	mg/kg	µg/g	µg/g	pg/mL	ng/hr	Units‡
0*	250	249	198	481†	0.681	0.739
1	250	245	189	615	0.355	0.777
0	500	276	186	474	0.466	0.864
1	500	254	189	685	0.532	0.829
Analysis of Variance – P values						
Nickel		0.16	0.64	0.0001	0.17	0.98
Magnesium		0.07	0.11	0.45	0.85	0.13
Ni x Mg		0.35	0.20	0.34	0.04	0.53
Pooled SD		30	13	0.19	0.276	0.18

* The basal diet contained 30 ng Ni/g.

† Data were transformed using the natural logarithm before analysis because they were not normally distributed; back-transformed data shown. Analysis performed on urine collected during wk 12.

‡ Units are (µ)moles *p*-nitrophenol formed/min/mL plasma × 10.

A mild magnesium deprivation resulted in changes that would suggest that it is beneficial, not detrimental, to bone health. Bone concentrations of minerals associated with bone growth and maintenance, calcium, manganese, and possibly phosphorus were increased by the mild magnesium deprivation. However, these bone composition changes apparently were not particularly beneficial to femur strength and shape because they were not affected by mild magnesium deprivation. Also, plasma alkaline phosphatase activity was not increased by magnesium deprivation. These findings do not preclude the possibility that a mild magnesium deprivation might adversely affect bone structure or strength. Another long-term mild magnesium-deficiency experiment found that trabecular bone was decreased while cortical bone and calcium concentration were increased in long bones *(10)*. The changes in amount of trabecular and cortical bone might be the basis for the changed calcium concentrations. The decreased trabecular bone in cancellous bone might eventually lead to adverse effects such as vertebrae crushing, as found in osteoporosis. Nickel deprivation did not enhance the effects of a mild magnesium deprivation on measured bone characteristics.

Nickel deprivation decreased femur strength. Because nickel did not affect bone calcium and phosphorus concentrations, urinary calcium excretion, and plasma alkaline phosphatase activity, it is unlikely that the change in strength was caused by increased bone density or mineral content. The change in bone strength might have been caused by a change in bone shape induced by nickel deprivation. The decreased ventral depth and moment of inertia (effect of bone geometry on resistance to bending) support this suggestion. The mechanism through which nickel apparently affects bone shape is unclear. However, it apparently is not through an effect on magnesium metabolism because nickel deprivation increased tibia magnesium in magnesium-adequate rats but did not significantly affect the tibia magnesium concentration in magnesium-deficient rats. Dietary magnesium did not influence the effect of nickel deprivation on bone strength and shape. Perhaps, as with urinary phosphorus excretion, the bone strength and shape changes might be indicating an effect of nickel on lipid metabolism or signaling function. Nickel deficiency reduces long-chain polyunsaturated fatty acids (PUFA) amounts and the 20 : 4/18 : 2 ratio in erythrocytes *(36)*, which indicates PUFA changes in other tissues. In bone, changes in PUFA affect bone mechanical properties *(37)*. The finding that nickel deprivation decreased the prostaglandin E_2 concentration in urine supports the suggestion that nickel might affect PUFA metabolism. Cyclic nucleotides, whose function apparently might be affected by dietary nickel *(31)*, apparently are involved in the regulation of skeletal remodeling *(38)*.

In summary, decreased kidney magnesium concentration and urinary magnesium excretion and increased urinary nitrate excretion indicate that feeding 250 mg Mg/kg diet for 13 wk produced a long-term mild magnesium deficiency in rats. The mild magnesium deficiency affected calcium metabolism or utilization, as evidenced by decreased urinary calcium excretion and increased tibia calcium concentration. The change in calcium metabolism or utilization, however, did not result in changes in femur shape or strength. Nickel deprivation had little effect on the response to mild magnesium deprivation and, thus, is not a stressor of magnesium need.

Decreased kidney nickel concentration and urinary nickel excretion and increased plasma triglycerides and tibia magnesium indicate that rats fed 30 ng Ni/g diet were nickel deficient. Nickel deficiency did not affect tibia calcium concentration or urinary calcium excretion, but increased urinary phosphorus excretion. Bone-breaking variables maximum force and moment of inertia also were decreased by nickel deprivation. Decreased moment of inertia in addition to decreased outside ventral depth of the femur suggests that nickel deprivation affected bone strength through changing bone shape rather than by changing bone density or mineral content. The finding that dietary nickel deprivation affected the urinary excretion of phosphorus and prostaglandin E_2 and plasma triglyceride concentration suggests nickel deprivation might have changed bone shape

and strength through an effect on lipid metabolism. The lack of interaction between nickel and magnesium in bone biomechanical measures indicate that nickel did not affect bone shape and strength through an effect on magnesium utilization or metabolism.

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